Serial Number: 10/813,482 Filing Date: March 30, 2004

Title: METHOD TO ENHANCE AGROBACTERIUM-MEDIATED TRANSFORMATION OF PLANTS

REMARKS

Reconsideration and withdrawal of the objection to and rejections of the claims, in view of the amendments and remarks herein, is respectfully requested. Claims 57 and 62 are amended, and claims 66 and 69-70 are canceled. The amendments are intended to advance the application and are not intended to concede to the correctness of the Examiner's position or to prejudice the prosecution of the claims present prior to amendment, which claims are in a continuing application of the above-referenced pending application. Claims 57-65, 67-68 and 71-76 are now pending in this application.

Claims 57 and 62 were objected to as being drawn to non-elected inventions. The amendment to claims 57 and 62, to recite that the one or more agents are sulfhydryl-containing agents, obviates the objection to claims 57 and 62.

Claims 58-66, 68 and 71-74 are rejected under 35 U.S.C. § 102(b) as being anticipated by Perl et al. (<u>Biotechnology</u>, <u>14</u>:624 (1996)) and claims 57-60, 62-67, 71-73, and 75-76 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Enrique-Obregón et al. (<u>Biotechnologia Aplicada</u>, <u>14</u>:169 (1997)). These rejections are respectfully traversed.

Perl et al. disclose that short exposures of diluted cultures of *Agrobacterium* to embryogenic calli of *Vitis vinifera* cv. Superior Seedless grape result in plant tissue necrosis and subsequent cell death (abstract) (the Examiner notes that grape is a dicot plant at page 5 of the Office Action dated March 13, 2007). To determine the effect of various antioxidants on necrosis, Perl et al. added antioxidants to the solid co-cultivation medium (treatments 1-6 in Table 1). Perl et al. relate that the presence of polyvinyl pyrrolidone (PVP), cysteine, ascorbic acid, or citric acid in the solid co-cultivation medium was unable to reduce necrogenesis, while the presence of dithiothreitol (DTT) or polyvinyl polypyrrolidone (PVPP) in the solid co-cultivation medium reduced browning to some extent but did not completely inhibit the phenomenon (page 625). Note that Perl et al. report that the presence of cysteine, a sulfhydryl containing agent, in the solid co-cultivation did not reduce necrogenesis. As the results for treatments 1-6 were obtained 48 hours after calli were transferred to solid media supplemented with a test agent, the results do not evidence how the presence of those agents in solid co-cultivation media may alter the stable transformation of plant tissue.

Filing Date: March 30, 2004

METHOD TO ENHANCE AGROBACTERIUM-MEDIATED TRANSFORMATION OF PLANTS Title:

Dkt: 600.479US2

Perl et al. also relate that an optimal effect in blocking necrogenesis was obtained with a double-layer medium containing PVPP and DTT, but that necrosis was not blocked when a double-layer medium with PVP, ascorbic acid, or cysteine in the solid medium, with or without DTT in the liquid medium, was employed (page 625). It is disclosed that stably transformed grape was obtained after co-cultivation of grape callus with PVPP for 48 hours, followed by incubating the callus in a double-layer medium with PVPP in the solid layer and DTT in the liquid layer for 7 days (Figure 3).

The Examiner is requested to consider the Rule 132 Declaration enclosed herewith, executed by Dr. Paula Olhoft, one of the co-inventors of the present invention. In the Declaration, Dr. Olhoft points out that the 2 day co-cultivation step in Perl et al. allows for Agrobacterium infection of the plant tissue and that the 7 day culture step in Perl et al. is intended to favor plant tissue growth prior to selective pressure. Dr. Olhoft also points out Perl et al. disclose that the combination of PVPP and DTT (which only occurred after co-cultivation) was found to improve plant viability, and that those agents inhibited tissue necrosis but did not affect Agrobacterium virulence.

Thus, the only protocol disclosed in Perl et al. to prepare transformed plants is the use of PVPP in solid co-cultivation medium followed by cultivation in a double-layer medium containing PVPP in the solid medium and DTT in the liquid medium.

Accordingly, withdrawal of the § 102(b) rejection is respectfully requested.

Enriquez-Obregón et al. report on the effect of three antioxidants on the growth of Agrobacterium in sugarcane. It is disclosed that a combination of ascorbic acid (15 mg/L), cysteine (40 mg/L) and silver nitrate (2 mg/L) was added to the precoculture liquid medium and the solid medium. After 3 days on solid media, explants were placed on selective media and the number of transformants determined (Table 2). It is disclosed that an efficient regeneration technique results in transgenic plants from the transformed explants, however, no data on those plants is provided in the Enriquez-Obregón et al. article.

In the final Office Action, the Examiner asserted that given the recognition of those of ordinary skill in the art in the value of transforming a sugarcane plant to improve the plant's agricultural yields and industrial production as taught by Enriquez-Obregón et al., one of skill in the art would be motivated to use the method of Enriquez-Obregón et al. for transforming

Filing Date: March 30, 2004

Title: METHOD TO ENHANCE AGROBACTERIUM-MEDIATED TRANSFORMATION OF PLANTS

Page 7 Dkt: 600.479US2

sugarcane and to optimize process parameters by varying the cysteine concentration and for transforming of other monocot plants, such as maize, wheat or rice, because one of ordinary skill in the art recognizes that a transformation procedure that works for one member of a group will also work for other members of the group.

In the Amendment filed on January 7, 2008, Applicant traversed the assertion that "one of ordinary skill in the art recognizes that a transformation procedure that works for one member of a group will also work for other members of the group" as a form of Official Notice for making a conclusory statement without support of a reference, and requested a reference to support the assertion or an affidavit of personal knowledge by the Examiner, pursuant to M.P.E.P. § 2144.03, in the next official communication.

In response to the request for a reference or an affidavit, in the Advisory Action dated February 5, 2008, the Examiner points to Applicant's claims 75 and 76. It is Applicant's position that the Examiner has not met his burden of supplying adequate evidence that any transformation procedure that works for one member of a group of plants will also work for other members of a group. "If applicant adequately traverses the examiner's assertion of official notice, the examiner <u>must</u> provide <u>documentary</u> evidence in the next Office action" (M.P.E.P. 2144.03(C).

With regard to the disclosure in Enriquez-Obregón et al., the Examiner is requested to consider the Rule 132 Declaration enclosed herewith. In that Declaration, Dr. Olhoft states that when any agent is added to plant media in an effort to improve outcome, there is a balance between plant cell viability and agent toxicity, for instance, at one concentration, the agent may not substantially impact plant cell viability while at a higher concentration the agent may decrease plant cell viability. Dr. Olhoft concludes that in view of the balance between plant cell viability and agent toxicity, and *Agrobacterium*-mediated virulence, there would be <u>no reason</u> to try higher amounts of any of the agents in the combination disclosed in Enriquez-Obregón et al. because of potential cytotoxicity.

Therefore, withdrawal of the § 103(a) rejection is respectfully requested.

Serial Number: 10/813,482 Filing Date: March 30, 2004

Title: METHOD TO ENHANCE AGROBACTERIUM-MEDIATED TRANSFORMATION OF PLANTS

CONCLUSION

Applicant respectfully submits that the claims are in condition for allowance and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicant's attorney (612) 359-3270 to facilitate prosecution of this application.

If necessary, please charge any additional fees or credit overpayment to Deposit Account No. 19-0743.

Respectfully submitted,

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Date 4/1/2008

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CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being filed using the USPTO's electronic filing system EFS-Web, and is addressed to: Mail Stop RCE, Commissioner of Patents, P.O. Box 1450, Alexandria, YA 22313-1450 on this

13x day of April 2008.

Name

Signature